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Morphological, molecular investigation of fungi isolated from layers poultry and their environment with their mycotoxins profile in Wasit province

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A total of 150 samples were collected from seven layers poultry farms IN Wasit Province of Iraq. Fifty (50) from layers poultry and hundred (100) from farm premises including poultry feed, surfaces associated with feed container and water drinkers indicating isolation of 60 (40%) mold and yeast based on morphological characteristics and diagnosed to species by using sequencing of the internal transcribed spacer region. *Aspergillus versicolor* recorded higher isolation rate 20/60 (33.3%), followed by *Candida albicans* 16/60 (26.6%), *Aspergillus wlwitschiae* 12/60 (20%), *Diutina catenulata* 10/60 (16.6%), and the lowest percentage was observed for *Lichtheimia ramose* 2/60 (3.3%). The analyses of Mycotoxins in poultry feed conducted by Enzyme Linked Immunosorbent Assay (ELISA), revealed aflatoxins B1 (AFB1) in all feed samples (70) with total range (6.945 ppb), and for each: soybean (8.060 ppb), corn (3.287 ppb) and wheat (3.287 ppb). While 60 (85%) out of 70 sample were contaminated with ochratoxin in feed samples with total range (2.658 ppb), for soybean, corn and wheat as (1.122 ppb), (2.596 ppb) and (1.068 ppb) respectively (permissible ratios <20 ppb). Also about 14 (57%) out of 70 samples were contaminated with T2 toxin with total feed range (33.629%) as (113.321 ppb) for corn, (99.817 ppb) for soybean and (64.486 ppb) for wheat samples (permissible ratios <100). This study indicates the importance of continuous mycological evaluation of poultry feed production, hen's fungal infections and Mycotoxins contamination may directly threaten the industry and harm the consumers at the same time.
Keyword: Morphological, molecular, layers poultry, mycotoxins profile, wasit province, Iraq.

INTRODUCTION

Fungal pathogens are a major threat to both human and animal health on a global scale (EL-Sheekh *et al.*, 2021). Even in the case of ideal cultural circumstances, harvest, storage, and handling were used, fungi, bacteria, and their toxins are inherent pollutants of the environment, particularly foods (Kabak *et al.*, 2006). Many different types of fungi can be found in soil, decomposing organic matter, animal feed, stored grains, and other materials. These fungi can cause spoilage and produce harmful poisons (Liu *et al.*, 2000 and Seyedmousavi *et al.*, 2015). The growth and dispersion of fungi are influenced by various environmental factors, including temperature, pH, moisture, degree of aeration, and amount and kind of nutrients (Alsohaili and Bani-Hasan., 2018). Isolation and identification of fungi from environmental sources is necessary for the identification of additional species, revising scientific classification, and assessing their effects on nature (Blakwell., 2022). A small number of fungus, particularly *Aspergillus spp*., causes frequent diseases in bird species (Ulrikh and Smolovskaya, 2021). the cause of Aspergillosis is a fungus-based illness that can affect humans and a variety of animal species (Maheswari and Komalavalli., 2013; Martins *et al.*, 2020). Public health could be at risk from the potential spread of mycotoxins from diseased birds to meat and eggs (Ulrikh and Smolovskaya, 2021; Titilayo *et al.*, 2022).

The role of these secondary toxic metabolites and their fate in environments as well as in agriculture one of the important fields that are still vague (Sulaiman, 2021) Other species like *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nidulans*, and *Aspergillus terreus* may also be isolated from avian cases of Aspergillosis, though less frequently than *A. fumigatus* (Martin *et al.*, 2007).

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Molecular identification methods based on complete fungal DNA extraction allow for the evaluation and species-level identification of different fungal isolates (Landeweert *et al.*, 2003). When identifying fungi using molecular methods, the 18S rRNA gene's PCR-amplified region is sequenced using primers specific to each type of fungus (Monod *et al.*, 2005). Most toxic species belong to the genera *Penicillium, Aspergillus, Alternaria,* and *Fusarium* (Pitt and Hocking, 2009). According to many researchers (Hassan *et al.*, 2022) the most prevalent mycotoxins discovered in feed and food include aflatoxins, zearalenone, T2-toxin, deoxynivalenol, ochratoxin A, fumonisins, and patulin (Hussein *et al.*, 2001). The majority of chicken mycotoxicosis are brought on by consuming contaminated feed in low concentrations over an extended period of time. However, ingesting higher concentrations causes acute clinical symptoms linked to particular essential organs, the immune system, various aspects of avian physiology, and mortality (Mabbett., 2004). Chicks have often been one of the most important experimental models in different experiments, especially toxicological studies (Jiato *et al.*, 2008; Okoli *et al.*, 2006). The study was aimed to:

1. Isolate and identify fungi from infected lesions of layers poultry and (feed, tank water) morphologically and by molecular methods.
2. Using comparison and analysis of rDNA ITS sequences, some fungal strains were classified at the species level.
3. Using direct competitive analysis (ELISA) to assay Aflatoxin, Ochratoxin and T2 toxin in feedstuff of seven farms which were suffering from high chiken mortality rate and decrees in egg production in Wasit province.

MATERIALS AND METHODS

***Sample Collection*:** The study included a total of 150 samples, 50 from infected chickens as shown in (Fig. 1) lesions of layers poultry (N=50), and 100 environment samples (N=100) from feed, surfaces of feed container and water drinkers collected from seven farms in Wasit province during the period October 2021 to January 2022.

***Fungal cultures*:** Samples were cultured on plates containing Sabouraued dextrose agar (SDA) with Chloramphenicol 0.05 gm/liter. Incubated at 25°C on dextrose agar and examined for growth daily until 3 weeks of incubation after which plates showing no growth were deemed negative. All isolates were sub-cultured on Sabouraued dextrose agar and stored for further identification (Jasuja *et al.*, 2013).

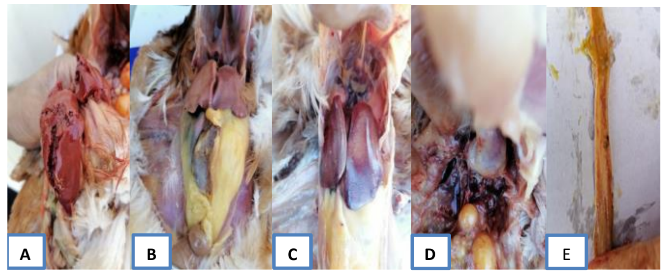

Figure 1. Postmortem lesions of diseased and freshly dead chickens described as white-yellow granulomas in pulmonary parenchyma and air sac membranes congestion of the lungs, rotten liver and yellow internal organs: A, B, C, D & E.

***Macroscopic and Microscopic Examination of Isolated Fungi*:** By examining the colony characteristics, the macroscopical fungal morphology was explored (color, shape, size and hyphae), microscopically a little piece of the mycelium put on Lactophenol cotton blue-stained slides and examined (Jasuja *et al.*, 2013).

Molecular Identification of Fungal Species*:** *DNA Extraction:** Using a DNA extraction kit from (ABIOpure, USA) The manufacturer's recommended method was followed to extract fungal genomic DNA from fungal growth. Primers: PCR primers have been designed in this study through the usage of NCBI-GenBank database. The primer set used to be composed of particular sense corresponding to the sequences as in Table -1

Table 1. Primers used in PCR amplification method (Palo *et al.*, 2022).

Primer	Sequence	Annealing temp. (°C)	Product size (bp)
ITS1	5`-TCCGTAGGTGAACCTGCGG-3`	55	500 bp
ITS4	5`-TCCTCCGCTTATTGATATGC-3`		

***PCR Amplification*:** PCR master mix reaction was prepared by using (GoTag Green Master Mix kit protocol). The PCR machine was configured for 30 cycles as shown in Table 2. PCR products have been visualized by agarose gel stained by Ethidium bromide dye (Biometra, Germany) show in Fig. 2.

Table 2. PCR program setting for fungal isolates.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	5 min	1
Denaturation	95°C	30 sec	
Annealing	55°C	30 sec	30
Extension	72°C	30 sec	
Final extension	72°C	7 min	
Hold	10	10 min	1

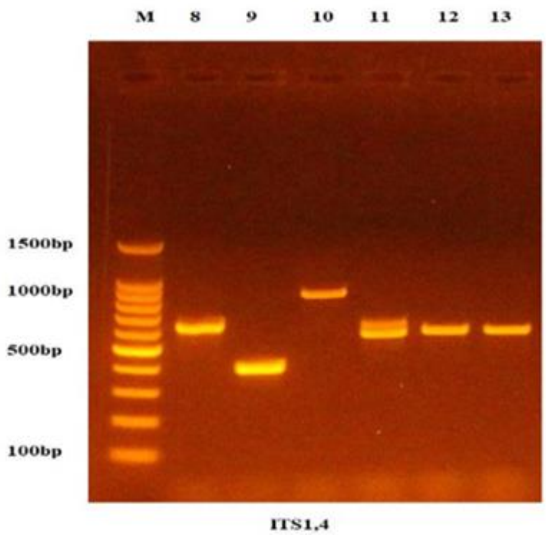

Figure 2. Agarose gel electrophoresis image shows PCR product analysis of fungal isolates. Lane M Marker ladder (100bp), lanes (8-13): ITS gene of Unknown fungal species with 500 bp.

***DNA Sequencing*:** PCR products were supplied to Macrogen Corporation - Korea for Sanger sequencing using automated DNA sequences from the ABI3730XL. The results were emailed to us, and we used specialized software to analyze them.

***Enzyme linked immuno-sorbent assay (ELISA)*:** A typical principle of direct competitive ELISA applied after extraction of mycotoxins from feeder, corn, soybean and wheat samples (Palo *et al.*, 2022).

RESULTS

***Fungal distribution in layers poultry and their environment*:** As it is shown in Table 2, sixty (60) fungal isolates including (5) species were recovered from totally (150) samples collected with a prevalence rate for *Aspergillus versicolor* 20/60 (33.3%) distributed as 4 isolates from Layers poultry lesion (20%), Poultry feed 9 (45%) while from surfaces associated with feed container 7 (35%). Isolates of *Candida albicans* were recorded 16/60 in rate of (26.6%) as 5 isolates from surfaces associated with feeder container (31.2%) and 11 from water (68.7%). Followed by *Aspergillus wlwitschiae* as 12 isolates (20%), 3 were from feeder (25%), 5 from surfaces associated with poultry feed container (41.6%) and 4 from water dispensers (33.3%). *Diutina catenulata* 10 (16.6%) were isolated from Layers poultry lesion 1 (10%), feeder 3 (30%), and water 6 (60%). While *Lichtheimia ramose* register the lowest percentage as 2 (3.3%) from feeder only**.**

Table 3. Distribution of isolated species from different sources included in the study.

Fungal species	No of isolates	Source								
		%	Layers poultry lesions	%	Poultry feed	%	surfaces of feed container	%	Water	%
Aspergillus versicolor	20	33.3	4	20	9	45%	7	35	-	-
Candida albicans	16	26.6	-	-	-	-	5	31.2	11	68.7%
Aspergillus wlwitschiae	12	20.0	-	-	3	25%	5	41.6	4	33.3%
Diutina catenulate	10	16.6	1	10	3	30%	-	-	6	60%
Lichtheimia ramose	2	3.3	-	-	2	100%	-	-	-	-
Total	60	100.0								

Table 4. Fungal isolates identified through sequencing of ITS region

Sr.	Fungal isolates	No. of isolates	%	Gen bank accession numbers	Identity (%)
1	*Aspergillus versicolor*	20	33.3	MH509421.1	100%
2	*Candida albicans*	16	26.6	KP675469.1	100%
3	*Aspergillus wlwitschiae*	12	20.0	MT318151.1	100%
4	*Diutina catenulate*	10	16.6	MT501155.1	100%
5	*Lichtheimia ramose*	2	3.3	KP132374.1	99%

Table 5. Concentration of different Mycotoxins in poultry feed samples

Mycotoxins	Feed sample	Soybean	Corn	Wheat	Unit	permissible ratios
Aflatoxins B1	6.945	8.060	3.287	3.287	Ppb	< 20
Ochratoxin	2.658	1.122	2.596	1.068	Ppb	< 20
T2 toxin	33.629	113.321	99.817	64.486	Ppb	<100

***Sequences Analysis*:** Using comparison and analysis of rDNA ITS sequences, five fungal strains were classified at the species level. The identically rate were emphasized and reported as is shown in Table 4.

***Mycotoxins Analysis*:** Aflatoxin, ochratoxin A, T2-toxin, and other contaminants in feed samples were quantitatively assessed using ELISA-based analytical test kits (21). Table 5 shows the distribution of aflatoxins B1 (AFB1) in all feed samples 70 (100%) with total range (6.945 ppb), and for each: soybean (8.060 ppb), corn (3.287 ppb) and wheat (3.287 ppb) (permissible ratios < 20 ppb). While 60 (85%) out of 70 sample were contaminated with ochratoxin in feed samples with total range (2.658 ppb), for soybean, corn and wheat as (1.122 ppb), (2.596 ppb) and (1.068ppb) respectively (permissible ratios < 20 ppb). Also about 14 (57%) out of 70 samples were contaminated with T2 toxin with total feed range (33.629%) as (113.321 ppb) for corn, (99.817 ppb) for soybean and (64.486 ppb) for wheat samples (permissible ratios <100).

DISCUSSION

Mycobiota and mycotoxins in chicken feed have been extensively studied during the past few years. The mycobiota of commercial chicken feed in Nigeria was examined in which their findings were summed up by isolation of *Aspergillus* spp. as the highest percentage which matches our results also *Penicillium* spp., *Mucor* spp., *Rhizopus* spp., *Epicoccum* spp., *Gymnoascus* spp., *Cladosporium* spp., *Mortierella* spp., yeast, and different bacterial isolates (Okoli *et al.*, 2006). (Saleemi *et al.*, 2010) also noted that Aspergillus species were the most prevalent fungal in Pakistan, followed by *Penicillium*, *Fusarium*, and *Alternaria*. Among the *Aspergillus* isolates, The most frequently isolated species were *A. niger* (37.74%), followed by *A. flavus* (22.64%), *A. ochraceous* (16.98%), *A. parasiticus* (13.21%), *A. carbonarius* (3.77%), *A. fumigatus* (3.77%), and *A. oryzae* (1.89 %). There were 73.58 % toxigenic fungus among Aspergillus isolates. Fourteen moulds genera from poultry feed in Iraq. Aspergillus, Penicillium, Rhizopus, Mucor, Alternaria, Scopulariopsis, and Eurotium were the most prevalent fungi, isolation of Aspergillus species recorded at 39.02% among toxigenic fungi, the same study report contamination of poultry feedstuff with Aflatoxin B1, B2, G1, G2 and Ochratoxin A in 79 from 90 samples at rate of 87.77% (Khalifa *et al.*, 2022).

The findings of the current investigation regarding with the percentage of moulds and yeasts were incompatible with that of (Greco *et al.*, 2014) who isolated *Fusarium* as the most frequent mycotoxigenic fungi (69.6%), followed by *Eurotium* (52.2%), *Penicillium* (45.65%), and *Aspergillus* (43.5%) (Castillo *et al.*, 2004). The occurrence of opportunistic yeast and fungi in different types of samples reflects the ability of the microorganism to colonies these biological niches in suitable conditions and the potential ability of these microorganisms to cause opportunistic infection affect economic and public health aspects (Bibani *et al.*, 2019).

One of the most crucial tools for identifying fungal species isolated from environmental sources based on the ITS rDNA region sequencing, additionally, as an advancement over traditional identifications (Kehinde *et al.*, 2014). The ITS region sequencing was used for DNA barcoding, which was used for molecular identification. Five species were identified using DNA barcoding with an identity range between 99 – 100 %. Due to their widespread distribution, ITS rRNA genes make great candidates for the phylogenetic study, also the fact that ITS rRNA genes are found in all organisms makes them practical method for phylogenetic research (Monge *et al.*, 2012).

One of the main risks to human and animal health is caused by fungus contamination of animal feed and the subsequent development of mycotoxins (Anderson *et al.*, 2007). The present results percentages of the Mycotoxins in feed are incompatible with a study done by (Greco *et al.*, 2014) in which Aflatoxins and ochratoxins were found in 91.1 % of the samples, fumonisins were found in 51.1 %, and T2-toxin was found in 2.2 %. While it was compatible with (Bibani *et al.*, 2019) where they analyzed Aflatoxin, Ochratoxin and T2-toxin in broiler's local and imported feeds and recorded Aflatoxin and T2-toxin as the most contaminant mycotoxin while the prevalence of ochratoxins contamination revealed a non-significant (P>0.05) value reflecting mismatching with our results according T2-toxin as the lower contaminant with total feed range (33.629%) .where T2- toxin is the causative agent of mouth and intestinal lesions in addition to declining egg production, weight loss and altered feather patterns, hysteroid seizures or an impaired righting reflex (Abidin *et al.*, 2011). As the results recorded by (Khalaf *et al.*,2015) when examining chicken feed in Nineveh according to Aflatoxin contamination by immunochromatographic test strips showed that only 15% sample feeds were positive hence the samples were chosen randomly from the feed available in the market, this contradicts the results of the current study in which all examined poultry feed samples were contaminated with Aflatoxin hence the included farm (Alsohaili *et al.*, 2018). The preharvest and/or postharvest phases are when the raw materials become contaminated by toxicogenic fungi, whereas during the stages of production, processing, shipping, and storage, finished feeds are exposed. Mold and mycotoxins have been found to vary in presence according on seasonality and geographic location. Temperature and humidity are crucial factors in both the growth of fungi and the formation of mycotoxin (Bibani *et al.*,2019; Michael *et al.*, 2006). Moisture content may consider as an important factor also of poultry feed and the other assets in the field with toxigenic fungi leading the mycotoxins to reach harmful

levels for human as consumer and animals (Gherbawy *et al.*, 2020).

***Conclusion*:** The isolation and identification of filamentous fungi from infected layers poultry, feed and surfaces of the containers in poultry farms in Wasit province displayed the presence of abundance an important pathogenic and toxicogenic fungi. When compared to traditional methods, the use of molecular approaches greatly improved the work's ability to identify between related species of fungi depending on analysis of rDNA ITS sequences. The ability of some types of fungi to secrete mycotoxins as it confirmed by ELISA test for fodder can be a major obstacle in the poultry industry Therefore, it is recommended to follow the periodic inspection of the feed.

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***Conflict of interest statement*:** The present study does not present any conflict of interests.

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***Ethical statement*:** All samples were collected according to authority given from a Institutional Animal care and use committee, University Medicine according to authority No: UM.VET.2021.066.

***Availability of data and material*:** All datasets generated or analyzed during this study are included in the manuscript.

***Code Availability*:** None

***Informed Consent*:** Written informed consent was obtained from the participants before enrolling in the study.

REFERENCE

Abidin Z, A. Khatoon and M. M. Numan. 2011. Mycotoxins in broilers: pathological alterations induced by aflatoxins and ochratoxins, diagnosis and determination, treatment and control of mycotoxicosis. World's Poultry Science Journal 27:485-496.

Alsohaili S, and B. Bani-Hasan. 2018. Morphological and Molecular Identification of Fungi Isolated from Different Environmental Sources in the Northern Eastern Desert of Jordan., Jordan Journal of Biological Sciences 11:329-337.

Anderson I.C. and, P.I. Parkin. 2007. PI. Detection of active soil fungi by RT-PCR amplification of precursor rRNA molecules. Journal of microbiological methods 68:248-253.

Bibani N.M.S., Z.K.Khidhir, A.S. Shaker and, S.M.S. Kirkuk. 2019. Analyses of mycotoxins in broiler's local and imported feeds. Iraqi Juournal of Veterinary Sciences 33:267-271

Bibani N.M.S., and Z.K. Khidhir, A.S.Shaker, M.S. Kirkuki and S.M. Abdulateef. 2019. Analyses of mycotoxins in broiler's local and imported feeds., Iraqi Journal of Veterinary Sciences 2:267-271.

Blackwell M. 2022. The Fungi: 1, 2, 3, 5.1 Million Species?. American Journal of Botany 98:426-438.

Castillo M.D., H.H.L.Gonz´alez, E.J.Mart´ınez, A.M.Pacin, and S.L. Resnik. 2004. Mycoflora and potential for Mycotoxins production of freshly harvested black bean from the Argentinean main production area.," Mycopathologia1 58:107-112.

EL-Sheekh M., M.Y.Bedaiwy, A.A.El-nagar, and E.W. El-gammal. 2021. EW. Isolation, identification and screening of cellulolytic activity of some fungi from different sources and localities in Egypt. Delta journal of Science 43:1012-5965.

Gherbawy Y.A., H.M.Elhariry, S.A. Alamri and E.G.A. El-Dawy. 2020. EGA. Molecular characterization of ochratoxigenic fungi associated with poultry feedstuffs in Saudi Arabia. Food Science & Nutrition 8:5298-5308.

Greco M.V., M.L.Franchi, S.L.R.Golba, A.G. Pardo, and G.N. Pose. 2014. Mycotoxins and Mycotoxigenic Fungi in Poultry Feed for Food-Producing Animals. The Scientific World Journal 8:5-9.

Hussein H.S. and, J.M. Brasel. JM. 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicologyv l167:101-134.

Jasuja N.D., R.Saxena, S.Chandra, S.C. Joshi. 2013. SC. Isolation and identification of microorganism from polyhouse agriculture soil of Rajasthan. African Journal of Microbiology Research 7:4886-4891

Jiatao X., F.U.Yanping, J.Daohong, L.I.Guoqing, H.Junbin.,H. Tom, and P. Youliang. 2008. P. Intergeneric transfer of ribosomal genes between two fungi. BMC Ecology and Evolution 8:87-96.

Kabak, B., A. D.Dobson, and I.I.L. Var. 2006. Strategies to prevent mycotoxin contamination of food and animal feed: a review. Critical reviews in food science and nutrition 46:593-619.

Kehinde M.T., F.Oluwafemi ., E.E. Itoandon, F.A. Orji and, O. Ajayi. 2014. Fungal Profile and Aflatoxin Contamination in Poultry Feeds Sold in Abeokuta, Ogun State, Nigeria. Nigerian Food Journal 1:73-79.

Khalaf B.S.S., N.M.A Younis, N.T.M.Sideeq, Y.S.M. Sultan and A.M. Shareef. 2015. Detection of broiler feeds contamination with Aflatoxins using rapid immunochromatographic test strips. Iraqi Journal of Veterinary Sciences 29:13-18

Khalifa E ., Mohesien M.T., Mossa M.I., Piekutowska M., Alsuhaibani A.M., Abdel-Wahab B.A., Sotohy S.A., Ghosh S., Helmy Y.A., Hussein M. and, A.M. Abdel-Azeem. AM. 2022. Diversity of Toxigenic Fungi in Livestock and Poultry Feedstuffs. *International Journal of Environmental Research* and *Public Health* 19:2-16

Landeweert R., Leeflang P., Kuyper T.W., Hoffland E, Rosling A., Wernars K., and E. Smit E.2003. Molecular identification of ectomycorrhizal mycelium in soil horizons. Appl Environ Microbiol 69:327-333.

Liu D., Coloe S., Baird R. and, J. Pedersen. 2000. J. Application of PCR to the identification of dermatophyte fungi. *Journal* **of** *Medical Microbiology* 49:493-497.

Mabbett T. 2004. Keep feeds free from fungi,”. African Farming 8:15-16. (doi: 10.1155/2014/968215).

Maheswari N.U. and R., Komalavalli. 2013. R. Diversity of soil fungi from Thiruvarur District, Tamil Nadu, India. International Journal of Current Microbiology and Applied Sciences 2:135-141.

Martin M.P., Bouck, K.P., Dykstra H.J., Wages, D.P. and H.J., Barnes. HJ. 2007. Disseminated Aspergillus flavus infection in broiler breeder pullets. *Avian Diseases* 51:626-631.

Martins A., M.David, A.Stevens, A.T. Lisa, C.V.R. Sabino and M.O. Xavier. 2020. Aspergillosis, Avian Species and the One Health Perspective: The Possible Importance of Birds in Azole Resistance. Microorganisms 8: 20-27. doi:10.3390/microorganisms8122037

Michael Z., L.R. John and B. Johann. 2006. Review of rapid methods for the analysis of mycotoxins., Mycopathologia161:261-273

Monge M., C.E. Magnoli and S.M. Chiacchiera. SM. 2012. Survey of Aspergillus and Fusarium species and their mycotoxins in raw materials and poultry feeds from Córdoba, Argentina. Mycotoxin Research 28:111-122.

Monod M., O. Bontems, C. Zaugg, B.L. Chenne, M. Fratti and R. Panizzon. 2005. Fast and reliable PCR /sequencing /RFLP assay for identification of fungi in onychomycoses. Journal of Medical Microbiology 55:1211-1216.

Okoli I.C., C.U. Nweke, C.G.Okoli. 2006. Assessment of the microflora of commercial poultry feeds sold in the humid tropical environment of Imo State Nigeria.,” International Journal of Environmental Science and Technology 3:9-14. doi: 10.1007/BF03325902.

Paloi, Soumitra, J. J. Luangsa-ard, W. Mhuantong, M. Stadler and N. Kobmoo. 2022. N. Intragey8pnomic variation in nuclear ribosomal markers and its implication in species delimitation, identification and barcoding in fungi. Fungal biology reviews 42:1-33 https://doi.org/10.1016/j.fbr.2022.04.002

Pitt J. and A. Hocking. 2009. Fungi and Food Spoilage., Springer, Berlin, Germany, 3rd edition.

Saleemi M.K., Khan M.Z., Khan A. and I. Javed. 2010. Mycoflora of poultry feeds and Mycotoxins producing potential of Aspergillus species. Pakistan Journal of Botany 42: 427-434. doi: 10.1155/2014/968215

Seyedmousavi S., J.Guillot, P.Arné, G.S .De Hoog. 2015. Aspergillus and aspergillosis in wild and domestic animals: A global health concern with parallels to human disease. Medical Mycolology 53:765-797.doi: 10.1093/mmy/myv067.

Sulaiman U. 2021. Influence of modern media on agricultural information dissemination: a study of poultry farmers in oyo state. Journal of Global Innovation in Agricultural Sciences 9:139-144.

Titilayo D.O.F., A. Neya, S. Bonkoungou, K. Dagno, A. Basso, A. L. Senghor. J. Atehnkeng, A. Ortega-Beltran and Ranajit Bandyopadhyay. 2022. Aflatoxin Contamination of Maize, Groundnut, and Sorghum Grown in Burkina Faso, Mali, and Niger and Aflatoxin Exposure Assessment. Toxins 14:1-14. doi.org/10.3390/toxins14100700.

Ulrikh, E. V. and O.V. Smolovskaya. O. V. 2021. Mycotoxins in fodder and its importance on safety of feed and the health of farm animals: a review. Online Journal of Animal and Feed Research11:219-223.